

In the Claims

1. (Currently Amended) An *in vitro* method for detecting the presence of a demyelinating disease diseases in an individual, for determining the stage or severity of said disease diseases in the individual, or for monitoring the effect of the therapy administered to an individual presenting said disease diseases, comprising:

a) detection detecting and/or quantifying quantification of the DUSP6 protein, of the dusp6 gene mRNA, or of the corresponding cDNA in a sample of said individual, and

b) comparing comparison of the DUSP6 protein amount, of the dusp6 gene mRNA amount, or of the corresponding cDNA amount detected in a sample of an individual, with the DUSP6 protein amount, with the dusp6 gene mRNA amount, or with the corresponding cDNA amount detected, respectively, in samples from control individuals or with normal reference values.

2. (Currently Amended) A method according to claim 1, wherein the demyelinating is selected from the group consisting of diseases are, among others, multiple sclerosis, Devic's syndrome, Baló disease, Marchiafava-Bignami disease, central pontine myelinolysis, acute disseminated encephalomyelitis, and or acute necrotizing hemorrhagic encephalomyelitis.

3. (Currently Amended) A method according to the previous claims claim 1, wherein said sample is selected from the group consisting of serum, urine, saliva, feces, and or cerebrospinal fluid.

4. (Currently Amended) A method according to claim 3, wherein the sample to analyze is obtained by any conventional method, preferably surgical resection.

5. (Currently Amended) A method according to claim 1, wherein the sample to analyze is obtained from an individual who has not previously been diagnosed with a demyelinating disease.

6. (Currently Amended) A method according to claim [[5]] 1, wherein the sample to analyze is obtained from an individual who has previously been diagnosed with a demyelinating disease, preferably multiple sclerosis.

7. (Currently Amended) A method according to claim 1, wherein the sample to analyze is obtained from an individual undergoing treatment, or who has been previously treated against a

demyelinating disease, preferably multiple sclerosis.

8. (Currently Amended) A method according to claim 1, further comprising characterized in that it comprises carrying out an extraction of the sample, either for obtaining to obtain a protein extract or for obtaining to obtain an extract consisting of total RNA.

9. (Currently Amended) A method according to claim 8, characterized in that detection the detecting of the DUSP6 protein comprises a first step of contacting the protein extract of the sample with a composition of one or more specific antibodies against one or more epitopes of the DUSP6 protein, and a second step of quantifying the complexes formed by the antibodies and DUSP6 protein.

10. (Currently Amended) A method according to claim 9, characterized in that said antibodies comprise a species selected from the group consisting of monoclonal antibodies, polyclonal antibodies, intact or recombinant fragments thereof, recombinant fragments thereof, of them, "combibodies," and Fab antibody fragments, and or scFv antibody fragments, specific against the DUSP6 protein; these antibodies being human, humanized or of non-human origin.

11. (Currently Amended) A method according to claims 9 or 10, characterized in that for quantifying the complexes formed by the antibodies and the DUSP6 protein[.]] are quantified using a technique techniques are used selected from the group consisting of formed by Western-blot, ELISA (Enzyme-Linked Immunosorbent Assay), RIA (Radioimmunoassay), Competitive EIA (Competitive Enzyme Immunoassay), DAS-ELISA (Double Antibody Sandwich-ELISA), immunocytochemical techniques, and immunohistochemical techniques, techniques based on the use of protein biochips, techniques based on the use of or microarrays including specific antibodies, assays based on precipitation with colloidal gold, in formats such as dipsticks; or by means of affinity chromatography techniques, ligand binding assays and or lectin binding assays.

12. (Currently Amended) A method according to claim 8, characterized in that the detecting detection of mRNA or corresponding cDNA dusp6 gene comprises a first amplification step of the mRNA included in the total RNA extract, or of the corresponding cDNA synthesized by reverse transcription of the mRNA, and a second quantification step of the amplification product of the mRNA or eDNA of the dusp6 gene.

13. (Currently Amended) A method according to claim 12, characterized in that the amplification

is carried out in a qualitative or quantitative manner by means of RT-PCR using oligonucleotide primers[[,]] wherein the sequences of the primers used to amplify the *dusp6* gene sequence are selected from the group consisting of being SEQ ID NO:1, and SEQ ID NO:2, and/or any other primer pair amplifying *dusp6* specifically.

14. (Currently Amended) A method according to claim 8, characterized in that ~~the detection of the~~ ~~detecting is carried out with~~ ~~of mRNA is carried out by specific probes of mRNA~~ or ~~the~~ corresponding cDNA specific probes of the *dusp6* gene by means of techniques such as, for example, Northern blot or Northern transfer.

15. (Original) A method according to claim 8, characterized in that mRNA detection is carried out by means of real time quantitative RT- PCR (Q-PCR).

16. (Cancelled)

17. (Currently Amended) An *in vitro* method for identifying and evaluating the efficacy of an agent ~~compounds~~ for therapy of demyelinating diseases, ~~preferably multiple sclerosis, said method~~ comprising:

- a) treating a primary culture of rat optic nerve oligodendrocytes with stimuli relevant to demyelinating diseases ~~to produce a culture of stimulated oligodendrocytes, preferably with excitotoxic stimuli such as Ampa or Kainate~~
- b) detecting and quantifying changes in the *dusp6* gene or DUSP6 protein expression in ~~culture cells of the culture of stimulated oligodendrocytes~~ in response to said stimuli,
- c) contacting the ~~pure~~ culture of stimulated oligodendrocytes ~~obtained in step a)~~ with the agent candidate compound under the conditions and for the time suitable for permitting interaction between the stimulated oligodendrocytes and the agent to form interacting stimulated oligodendrocytes them to interact,
- d) detecting and quantifying the *dusp6* gene or DUSP6 protein expression levels in the culture of interacting stimulated oligodendrocytes, and
- e) comparing the expression levels obtained in step d) with the corresponding levels in the culture cultures of stimulated oligodendrocytes not treated with the agent candidate compound.

18. (Cancelled)

19. (Currently Amended) Use A method ~~Method~~ for the treatment of the neurodegenerative phase of demyelinating diseases, ~~especially multiple sclerosis~~ which comprises the administration of an agent that inhibits DUSP6 protein expression and/or activity, or that inhibits the lethal effects of induction of DUSP6 protein expression, ~~in the manufacturing of a pharmaceutical composition for the treatment of the neurodegenerative phase of demyelinating diseases, especially multiple sclerosis.~~

20. (Currently Amended) Use Method ~~The method~~ according to claim 19 wherein said agent is selected from the group consisting of ~~formed by~~:

- a) an antibody, or combination of antibodies, specific against one or more epitopes present in the DUSP6 protein, ~~wherein said antibody comprises~~ preferably a human monoclonal antibody, ~~or a~~ humanized monoclonal antibody~~[[;]]~~ also being possible a fragment of the antibody, a single-chain antibody or an anti-idiotype antibody,
- b) cytotoxic agents~~[[,]]~~ selected from the group consisting of such as toxins, molecules with radioactive atoms, ~~and~~ ~~or~~ chemotherapeutic agents, which include, without limitation, small organic and inorganic molecules, peptides, phosphopeptides, antisense molecules, ribozymes, triple helix molecules, small interference RNA, double strand RNA, etc., inhibiting DUSP6 protein expression and/or activity, such as, for example, the ~~dusp6 specific antisense oligonucleotides SEQ ID NO:3 or SEQ ID NO:4, or any antisense oligonucleotide with an homology with said molecule exceeding 50%, or any~~ ~~dusp6 specific antisense oligonucleotide inhibiting its expression, and~~
- c) DUSP6 protein antagonist compounds inhibiting one or more of the DUSP6 protein functions.

21. (Currently Amended) Use Method ~~The method~~ according to claim 19, which comprises the additional administration of ~~wherein said pharmaceutical composition further contains another active ingredient, preferably one which inhibits a~~ DUSP6 protein function inhibitor.

22. (Original) A ~~dusp6 specific antisense oligonucleotide selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:4.~~

23. (Currently Amended) A kit for an *in vitro* method of detecting the presence of a demyelinating disease in an individual, said kit comprising that comprises an antibody that specifically recognizes the DUSP6 protein and a carrier in suitable packing.

24. (Currently Amended) A kit for an *in vitro* method of detecting the presence of a demyelinating disease in an individual, said kit comprising that comprises a primer pair designed to specifically amplify a nucleic acid having a sequence that is specific to the *dusp6* gene.

25. (Currently Amended) A kit according to claim 24, wherein the sequence of the primer pair is selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

26. (Currently Amended) A kit according to ~~anyone of claims 23 to 25~~ claim 23 that is employed to detect the presence of demyelinating diseases in an individual, to determine the stage or severity of said conditions in an individual or to monitor the effect of the therapy administered to the individual with said conditions.

27. (New) A method according to claim 8, characterized in that the detecting of corresponding cDNA *dusp6* gene comprises a first amplification step of the corresponding cDNA synthesized by reverse transcription of the mRNA, and a second quantification step of the amplification product of the cDNA of the *dusp6* gene.